TEST METHOD #28

DETERMINATION OF SOLUBLE F (IONIC)

SCOPE

This procedure is applicable to the simultaneous determination of sodium monofluorophosphate and ionic fluoride in dental cream.

PRINCIPLE

The method is based on a modification of liquid chromatography, combining ion exchange, eluent suppression and conductrimetric detection.

APPARATUS REQUIRED

Dionex Analytical Ion Chromatograph
Analytical Column: 3 x 250 mm Chromex Anion Resin
Suppressor Column: 6 x 250 mm Chromax Anion Suppressor Resin
Eluent: 0.003 N Sodium Bicarbonate
         0.002 N Sodium Carbonate
Flow: 230 ml/hr.

METHOD

A standard solution of MFP and sodium fluoride is used to calibrate the instrument. The sample is a 4% water soluble portion of a dental cream.

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #27

TITLE: DETERMINATION OF SOLUBLE F (IONIC)

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #27

DETERMINATION OF SOLUBLE F (IONIC)

APPARATUS REQUIRED

1. Orion digital pH meter model 701 or equivalent.
2. Orion Fluoride electrode model 94-09.
3. Orion reference electrode model 90-01 filled with equitransferent filling solution - Orion No. 90-00-01 or Calomel reference electrode.
4. Plastic beakers: 100 ml.

REAGENTS REQUIRED

1. Sodium Fluoride, Stock Solution: Weigh 1.1054 grams NaF (based on 100% purity) and dissolve in distilled water. Dilute to one liter. Store in plastic bottle. This solution is equivalent to 0.50 mg F/ml and is stable for approximately 3 months.

2. Ammonium Acetate Solution, 7.0M: Dissolve 539.6 grams ammonium acetate in distilled water and dilute to one liter.

STANDARD FLUORIDE SOLUTION

1. Pipet 10 ml of stock sodium fluoride into 100 ml volumetric flask and dilute to mark with distilled water. This solution is equivalent to 50 ug F/ml and is stable for approximately 1 month.

2. Using the solution from Step 1, prepare a minimum of two standards, one higher and one lower than the expected amounts in the sample. For example, if the expected value is 80 ppm, pipet in 1 ml into a 100 ml volumetric flask and add 20 ml of 7.0M Ammonium Acetate solution. Dilute to volume with water and mix. This solution is equivalent to 50 ug F/100 ml. Repeat using 2 ml to prepare a 100 ug F/100 ml solution.

PROCEDURE

1. Weigh 20.00 ± .02 grams dental cream into 150 ml beaker. Add 30 ml distilled water and mix with glass rod to disperse dental cream; rinse glass rod with distilled water and further mix on a magnetic stirrer.

2. Quantitatively transfer dental cream solution with distilled water to 100 ml volumetric flask and fill to mark. Mix on magnetic stirrer for 3-5 minutes or until solution has a creamy consistency.

3. Centrifuge a portion of the dental cream.

4. Pipet 5 ml of the clear centrifuged solution into a 100 ml volumetric flask. Add 20 ml 7.0M Ammonium Acetate, fill to mark with distilled water and mix well.

5. Place approximately 25 ml of the lower standard fluoride solution in a plastic beaker.

6. Place the electrode into the solution and set the meter to read expanded millivolt. Let solution stand for at least 5 minutes or until equilibrium between electrodes and solution is reached. (Change in millivolts in approx. 0.1 mv/min.) See Note A. Record value.

7. Remove electrodes from solution and rinse off with distilled water. Gently wipe electrodes dry using tissue paper.

8. Place electrodes in the higher concentration of standard NaF solution. Let stand in solution for at least 3 minutes. Read expanded millivolt value.

9. Repeat for samples.

CALIBRATION CURVE AND CALCULATION

1. Plot the log of the standard F concentrations vs. the expanded millivolts on linear graph paper. See Note B. Example: Log of 50 µg/100 = 1.699, log of 100 µg/100 ml = 2.000.

2. Extrapolate the log of the sample concentrations from the calibration curve and take the antilog to determine the ug of F/100 ml of each sample. Example: Antilog 1.903 = 80 ug/100 ml.

3. Calculate the ppm of fluoride ion using the following equation:

$$\frac{\mu g\ F/100}{\dfrac{gm.\ sample}{100}\ x\ 5/100} = \frac{\mu g\ F}{gm.\ sample\ x\ .05} = ppm\ F\ ion$$

Example: $\dfrac{80}{20\ x\ .05 = 1} = 80\ ppm\ F\ ion$

NOTES:

A. As check on the standard solutions and/or the electrodes, at this
point adjust the expanded millivolt value with the calibration knob
to correspond with the millivolt reading. Once adjusted do not change
settings for the rest of the analysis. The millivolt reading for a
given concentration of F ion should not change from day to day. Any
variation will indicate a change in the standard solution or a deteri-
oration of the electrode.

B. Semi-log paper can be used and the concentrations read directly from
the graph; however, the precision and accuracy of the method is better
when linear paper is used.

STANDARDS FOR FLUORIDE DENTIFRICES

TEST METHOD #28

TITLE: DETERMINATION OF SOLUBLE F (IONIC)

Recommended for the following systems:

a. Sodium Monofluorophosphate/Insoluble Sodium Metaphosphate

b. Sodium Monofluorophosphate/Dicalcium Phosphate

c. Sodium Monofluorophosphate/Alumina

d. Sodium Monofluorophosphate/Silica

e. Sodium Monofluorophosphate/Calcium Pyrophosphate

TEST METHOD #29

Title: Determination of Soluble Fluoride in Sodium Fluoride - High-Beta-Phase
Calcium Pyrophosphate Toothpaste Supernatants by Fluoride Electrode

Recommended for the Following System:

a. Sodium Fluoride - high-beta-phase calcium pyrophosphate

Determination of Soluble Fluoride in Sodium Fluoride - High-Beta-Phase Calcium Pyrophosphate Toothpaste Supernatants by Fluoride Electrode

Principle and Scope

This method is an empirical one, designed to provide a reproducible measure for control purposes of the concentration of soluble fluoride present under normal toothbrushing times and dilutions. A 3:1, water:product slurry is prepared in which the paste has been thoroughly and intimately dispersed and the slurry is centrifuged to give a clear supernatant. The method of supernatant preparation is critical and must be adhered to if analytical reproducibility is to be attained. Finally, the supernatant is diluted 1:1 with EDTA/THAM solution, and the fluoride concentration of this solution is determined by direct potentiometry with the Orion Fluoride Electrode. Emf is converted to "parts per million" (ppm) fluoride in the supernatant by means of an appropriate calibration curve.

Reagents	Suggested Types or Sources (equivalent items may be used)
Sodium fluoride	Baker's Analyzed Reagent, 99.5% minimum purity
Deionized distilled water	Laboratory supply
(Ethylene dinitrilo) tetraacetic acid, disodium salt ($Na_2EDTA \cdot 2H_2O$)	ACS Grade
2-Amino-2-(hydroxymethyl)-1,3-propanediol (THAM)	Matheson, Coleman & Bell, M.P. 170-171°C.
Sodium hydroxide solution	Approx. 5 N (dissolve 50 g NaOH pellets in distilled water and dilute to 250 ml)

Apparatus	
Pipettes	5-ml, 25-ml, 50-ml, Class S
Magnetic stirrer	Labline "Magne-Stir"
Stirring bars	Teflon-coated, 7/8-inch
Polyethylene bottles	250-ml, 500-ml
Volumetric flasks	50-ml, 100-ml, 250-ml, 500-ml, 1000-ml
Expanded-scale pH meter	Beckman Expandomatic
Fluoride-specific ion electrode (see Note 1)	Orion Research, Inc., 11 Blackstone Street, Cambridge, Mass. 02139 Model 94-09-00. (Available from Fisher Scientific Co.)
Single-junction reference electrode	Orion Research, Inc., Model No. 90-01
Glass electrode (for pH measurement)	Beckman or Corning
Saturated calomel reference electrode	Beckman or Corning

Electrode holder	Orion Research, Inc., Model No. 92-00-01
Centrifuge, high-speed	International Model CS, UV, 2N, or 2 EXD with high-speed attachment
Centrifuge tubes	25-ml, International No. 298
Plastic beakers	100-ml disposable
Tygon-tipped stirring rods	8-inch, with about a 2-inch piece of Tygon tubing slipped over one end, such that an inch of Tygon extends beyond the glass. The extended tubing is sliced to form four tines.
Graph paper	Codex Brand No. 31,286 (two-cycle semi-log graph paper)

Sodium Fluoride Stock Solution (250 ug F⁻/ml):

Dry about 1 g of sodium fluoride at 105°C for 3 hours. Store the sodium fluoride in a desiccator and allow to cool. Weigh out 0.2763 g of the dried reagent, dissolve in distilled deionized water, and dilute to 500 ml in a volumetric flask. Minimize the time of contact with glass. Store in a polyethylene bottle.

0.2 N EDTA/0.2 N THAM Solution (pH = 8.0):

Weigh out 74.4 g disodium EDTA into a 1000-ml beaker. Add 24.2 g THAM (2-amino-2-(hydroxymethyl)-1,3-propanediol) and about 800 ml of hot distilled water (90-100°C). Insert a magnetic stirring bar and stir. Insert the glass-calomel electrode pair (standardized with pH = 7.0 buffer) and adjust the pH meter temperature control to the temperature of the solution. Slowly add 5 N NaOH solution until the pH of the solution is 7.5-8.0. Stir until the solids are completely dissolved. Cool the solution, adjust the pH to exactly 8.0 by adding more NaOH as necessary, and transfer to a 1000-ml volumetric flask. Rinse the beaker several times with small portions of distilled water and add the rinsing to the flask. Finally, dilute to the mark and mix well.

Standard Calibration Solutions:

Prepare standard calibration solutions by carefully measuring the appropriate quantities of NaF stock solution (250 ug F⁻/ml), EDTA/THAM solution and water into the appropriate volumetric flasks as specified in the table below:

Solution No.	ppm F⁻	Volumetric	ml NaF Stock Solution	ml EDTA/THAM	ml H_2O
1	25	100 ml	5	50	dilute to mark
2	100	50 ml	10	25	dilute to mark
3	150	50 ml	15	25	dilute to mark
4	200	50 ml	20	25	dilute to mark
5	250	50 ml	25	25	---

These solutions contain 12.5 to 125 ppm F⁻, but they represent the range 25-250 ppm F⁻ in a sample supernatant diluted 1:1 with EDTA/THAM solution.

Temperature Control of Solutions:

In order to prevent the standardizing solutions and test supernatants from being heated by the magnetic stirrer during the potential readings, a section of cardboard should be placed on the stirrer and the beaker set on the cardboard. The potential of the standardizing solution and each test supernatant should be read at room temperature (70-80°F).

Standardizing the Potential Scale of pH Meter

The pH meter should be used in the expanded scale and -mv modes. The potential range necessary for fluoride activity measurements for this application is 0 to -200 mv.

The scale of the meter is standardized by adjusting it to read -100 mv (the center of the mv scale) when the fluoride and reference electrodes are in contact with the 250 ppm F^- calibrating solution (Solution #5). This standardizing procedure should be performed periodically (as noted below) in conjunction with emf measurements on both calibrating supernatants and on test sample supernatants to insure accuracy and reproducibility of results.

Before the electrodes are removed from a solution, the "stand-by" button on the meter must be pressed.

Response Check of Orion Fluoride-Specific Ion Electrode

The response of the fluoride electrode must be checked each time it is used in order to establish the potential response versus concentration of free fluoride. (This is to verify the slope and linearity of the electrode's response.)

The Orion single-junction reference electrode should be used as the reference electrode. (See Note 1.) The following two solutions are necessary and sufficient for checking the response of the electrode:

        Standard Calibration Solution #1   (25 ppm F^-)

        Standard Calibration Solution #5   (250 ppm F^-)

These solutions can be prepared from stock solutions as described earlier. The electrodes are first put into Standard Calibration solution #5 (250 ppm F^-) and the potential adjusted to read -100 mv (estimated to the nearest 0.1 mv) (see "Standardization of the Potential Scale of the pH Meter", above). Solutions #1 and #5 should then be read in that order; the potential of #5 should not drift more than $\pm$ 1 mv and should be adjusted to -100 mv in any case each time it is checked. The solutions should be sitrred during the emf measurement, but not so vigorously as to allow bubbles to form and touch the fluoride electrode membrane crystal. At least one minute should be allowed for the electrode to reach a steady potential. The potential difference between solutions #1 and #5 should be 59 $\pm$ 1 mv. If the electrode does not behave in this manner, it should be replaced by a new electrode.

Preparation of Calibration Curve

In order to determine fluoride levels in sample supernatants directly from the emf measurements as read on the pH meter, it is necessary to have a calibration curve which converts millivolts to ppm F^-.

Prepare the five standard solutions as described previously in the "Standard Calibration Solutions" section.

Before the fluoride level of a standard solution is measured, the meter should be adjusted to read -100 mv when the electrodes are equilibrated in Calibration Solution #5 (250 ppm F^-). The potential adjustment should be made after one minute of stirring to allow the electrodes to reach a steady potential. The electrodes should then be rinsed thoroughly with distilled water, especially the membrane crystal of the fluoride electrode, and gently wiped dry with absorbent tissue. The potential of each standard solution is then measured in the same manner as for Solution #5. The potential should be read to the nearest 0.1 mv and that value recorded. After each solution is measured, the electrodes are rinsed and dried and the potential of the 250 ppm F^- standard solution remeasured. If the potential is more than $\pm$ 1 mv from -100 mv, the meter should be readjusted to read -100 mv, and the potential of the standard solution remeasured. The emf's of the other calibration solutions are measured and recorded in the same way. (The technique of indexing the meter at -100 mv with a standardizing solution makes it possible to use the same calibration curve day after day and maximizes the precision and accuracy of the direct potentiometric electrode method.) The data are then plotted on semi-log graph paper.

The calibration curve shown in this procedure was generated in the manner described above, using the data from the following table:

ppm F^- (Standard Solutions)	Millivolts (from pH meter)
25	- 41.0
100	- 76.7
150	- 87.2
200	- 94.4
250	-100.0

Preparation of Supernatants

The analysis for soluble fluoride is done on supernatants prepared from slurries of toothpaste in the manner described below. The results are reported as ppm F^- in the _supernatant_, not the toothpaste.

Extrude and discard the first inch of toothpaste from the sample tube, then weigh on a top-loading, direct-reading balance of 0.01 g sensitivity 10.00 grams of the paste sample into a 100-ml beaker. Pipet 30 ml of distilled water into the beaker and then set a clock-timer for 5 minutes immediately before starting the slurrying. Using a Tygon-tipped stirring rod, gently disperse the paste sample in the water by rubbing and stirring to break up the paste phase as quickly as possible. This operation is continued for one minute at which time the paste should be thoroughly and intimately dispersed in the water to form a homogeneous slurry. (If a homogeneous slurry has not been produced after one minute of stirring, continue stirring until it is, but do not stir longer than two minutes. With a little experience an analyst will have no difficulty in thoroughly dispersing the sample within the one minute period.)

Two sample slurries can conveniently be prepared at the same time, if desired, in which case the 30 ml of water is added to each sample in quick succession, and each sample/water system is stirred with its own individual Tygon-tipped stirring rod as described above for about fifteen seconds successively and alternately for a period of two minutes. An intimately-dispersed homogeneous slurry of each sample must result.

Pour 20-22 ml of the uniform slurry into a 25-ml centrifuge cup, balance very precisely on a 2-pan Harvard trip balance with a second cup containing water or a second sample slurry. Five minutes after starting to slurry the pastes (by the clock-timer), start the centrifuge and spin the slurries for a minimum of 30 minutes at 11,000 r.p.m. If the centrifuge has the capacity, two other pastes may be slurried at this time, and the centrifuge may be stopped to add these two samples at the end of their 5-minute time period. All four samples are then centrifuged for 30 minutes. After centrifuging, the clear supernatant should be carefully decanted into a labeled vial.

CALIBRATION CURVE FOR SOLUBLE FLUORIDE
IN SUPERNATANT FROM SODIUM FLUORIDE – HIGH-BETA-PHASE CALCIUM PYROPHOSPHATE DENTIFRICE SLURRY

(Diluted 1:1 with EDTA/THAM)
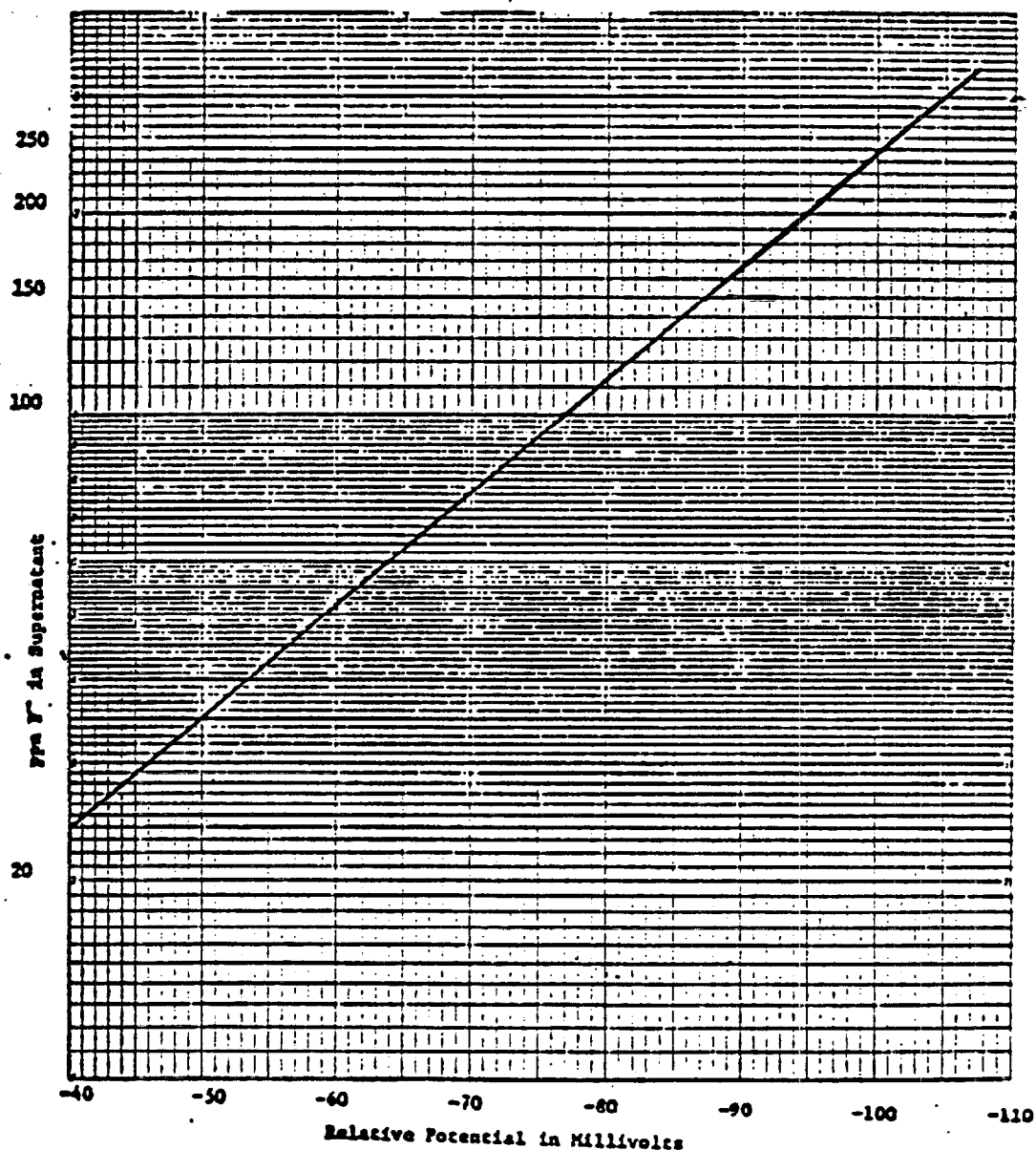
Test Samples

The soluble fluoride value for a test sample of sodium fluoride – high-beta-phase calcium pyrophosphate dentifrice is determined by first preparing a supernatant from the sample, as described above. Then pipet 10 ml of the supernatant into a 100-ml plastic beaker containing a stirring bar. Pipet 10 ml of EDTA/THAM solution into the same beaker and stir on a magnetic stirrer. Measure the potential of this diluted supernatant following exactly the same procedure described for the standard calibration solutions in the section "Preparation of Calibration Curve". The soluble fluoride value of a sample, as parts per million F^- in the supernatant (not the toothpaste) is found by using the calibration curve to convert from millivolts, as read on the pH meter, to ppm F^-.

It is very important that the response (linearity) of the electrode be checked daily and that the pH meter be standardized frequently while samples are being tested.

Note

1. An Orion Fluoride-Specific Ion Combination Electrode, Model No. 96-09, may be used to replace the fluoride-specific ion electrode and single-junction reference electrode listed in the "Apparatus" section of this method.

2. The adjustment of the pH meter to-100 MV (the middle of the scale) is a convenient but arbitrary setting. This potential is near the limit of adjustment, and some pH meters may not be able to be adjusted to -100 MV. If this is the case, another setting (i.e. -90MV) should be used. In either case, the meter should be adjusted to the same starting potential with calibration solution #5 (250 ppm F^-) before the potential of a sample is read.

TEST METHOD #30

Title: Determination of pH by Glass Electrode Method

Recommended for the Following Systems:

a. Stannous Fluoride - calcium pyrophosphate

b. Stannous Fluoride - silica

c. Sodium Fluoride   - high-beta-phase calcium pyrophosphate

Determination of pH by Glass Electrode Method

Material	Concentration	Temp. °C.	Special Instructions
Toothpaste	25%	25 ± 2	Equipment: (1) Timer, (2) Tygon policeman made by making 1-inch slits in tubing, starting at the end of the tubing. Operation: Weigh the water and paste for a 25% paste slurry at 25%°C. Start the timer as soon as paste and water are combined, and mix to a uniform slurry with the policeman. Immerse electrodes in the slurry at 60 seconds, and turn on pH meter at 70 seconds. Measure pH at 90 sec. Check pointer needle alignment, and if not exact, adjust and repeat pH measurement.

Reagents

Buffer Solutions

Use standard buffer solutions to cover the pH range desired, such as 4, 7, 9 and 10. The instrument should be set to read (or adjust) to the pH of the buffer solution which is stated by the manufacturer as correct at the temperature prescribed. If a working buffer solution is prepared in the laboratory, it should be checked against a standard buffer, either Coleman or Beckman. Beckman buffer solutions are recommended. Each pH range gives the corrected pH value at different temperatures. Several pH ranges are available in bulk quantities. Neutral or acid buffer solutions may be stored in stoppered Pyrex glass. Alkaline buffer solutions (pH 8 or higher) should be stored in stoppered inert plastic containers (polyethylene or polystyrene) or glass coated with an alkali-resistant material, such as paraffin wax.

Neutral distilled water

Boil distilled water vigorously for 15 minutes or longer, and cool rapidly while aerating with carbon dioxide-free air or cylinder nitrogen. Expulsion of CO_2 may be accomplished by prolonged aeration alone without boiling if desired. The air or nitrogen is dispersed into fine bubbles by passage through a fritted disc such as Harshaw H-27020. The pH of water so prepared should be between 6.8 and 7.2 at 25°C. when determined in a closed container under a blanket of nitrogen. Store in Pyrex glass with rubber, not cork, stoppers. Protect against CO_2 with an Ascarite or soda-lime tube to permit withdrawals.

Deionized water may be substituted. Such water must be protected from CO_2 in the same way that deaerated, distilled water is protected.

pH Meter Beckman Laboratory Model G, N-1, N-2, or Zeromatic pH meters or Leeds & Northrup Universal or Portable pH Indicator Assembles, or Corning 7 and 10 have been found satisfactory. Meters from other, newer suppliers may be used if equivalent to models listed.

| | | | Electrodes: Beckman | | | | | Instrument | |
Old Part No.	New Part No.	Type	Elec. Length	Lead Length	pH Range	Temp. Range °C	G	N-1;N-2;H-2 Zeromatic	
1190-42	40485	Glass	5"	30"	0-11	5 to 50	X		
1190-80	50598	Glass	5"	30"	0-11	-5 to 80	X		
4990-80	41262	Glass	5"	12"	0-11	-5 to 80		X	
4990-83	41263	Glass	5"	30"	0-11	-5 to 80		X	
1190-72	40495	E-2 Glass	5"	30"	0-14	15 to 80	X		
4990-73	41260	E-2 Glass	5"	30"	0-14	15 to 80		X	
or Corning	476022	Entire pH Range	5"	30"	0-14	15-80			
1170	39170	Calomel Fiber	5"	30"	-	-5 to 90	X	X	
Corning	476002	Calomel Fiber	5"	30"	-	-5 to 90	X	X	
1170-71	40463	Calomel Sleeve	5"	30"	-	-5 to 90	X	X	
4970	39970	Calomel Fiber	5"	12"	-	-5 to 90		X	
4970-71	41240	Calomel Sleeve	5"	12"	-	-5 to 90		X	
8970-2	43462	Cal. Invert. Sl.	5"	30"	-	-5 to 90	X	X	

Electrodes from other, newer suppliers may be used if equivalent.

Notes:

1. 12" leads should be used only if 30" leads are not readily available.

2. Fiber-type reference electrodes are satisfactory for clear solutions. For turbid solutions or slurries (where particles may clog the fiber) sleeve type electrodes are recommended.

3. Keep the KCl solution in the body of reference electrodes saturated at all times. One or two crystals should be present. A heavy deposit of KCl crystals may interrupt diffusion and should be avoided. The liquid level should be kept within 1 to 2 cm. of the filling opening.

4. Maintain a spare set of the most frequently used electrodes ready for use. (Valuable information about the preparation, care, maintenance and testing of electrodes is given by the manufacturer in the leaflet that comes with the electrodes.)

5. Data above is from Beckman Bulletin 86L. For Leeds and Northrup instruments, use the electrodes supplied with the instrument.

6. In solutions where sodium ion concentration exceeds 0.1 N and pH is over 9.5, Beckman E-2 glass electrodes are not accurate. Apply a proper correction.

7. Silicone rubber replacement sleeves for Beckman reference electrodes are available. They are not recommended since in some applications they have given erroneous results.

8. To renew the batteries in Beckman Model G pH meter use Burgess "A" No. 4FH, 1.5 volts; "B" No. 5156, 22-1/2 volts; "C" No. 5540, 7.5 volts; or their equivalent. Batteries with a metal case such as Eveready No. 735-NEDA 900 may cause instability in some instruments and should not be used if this occurs.

SODIUM - ION CORRECTIONS OF TYPE "E" GLASS ELECTRODES AT 25° C
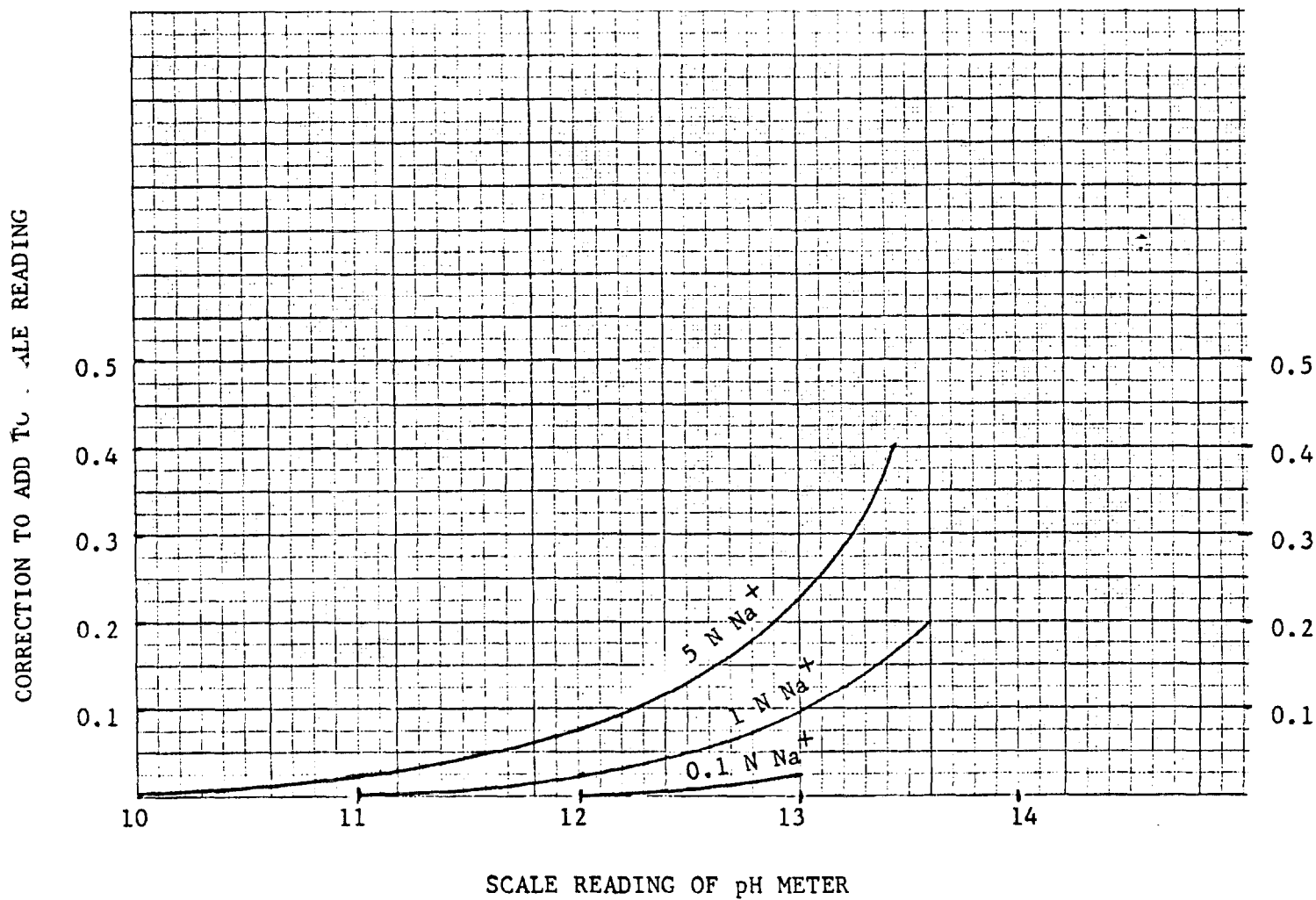
Operation

Follow manufacturer's instructions to operate the meter. Check at least two buffer solutions of different values, such as 4 and 10, when the meter is first turned on by standardizing the meter on one buffer and reading the second. The pH of the second buffer solution should check within 0.05 of the given value. (If poor agreement between buffers is found, check for old or contaminated buffer solutions, faulty electrodes or improper temperature setting.) Subsequent checks against standard buffer solutions should be made at least twice on each day on which pH measurements are required. Choose buffer solutions as close as possible to the pH range of the sample to be tested. The pH varies with temperature, the variation being greatest with high pH buffers. In checking standard buffer solutions, the temperature should be brought to that for which correct pH is given.

After adjustment of the instrument, place a suitable quantity of the slurry to be tested in a beaker and adjust to prescribed temperature. Enough sample should be used to cover electrode tips completely. Lower the electrodes into position and stir with the mechanical agitator while measuring the pH. Agitation should be vigorous enough to mix the slurry thoroughly without whipping air into it.

After each usage the electrodes should be washed free from the sample with distilled water. Remove any excess water by touching with a clean piece of absorbent tissue, such as Charmin or equivalent. When not in use keep electrode tops immersed in pH 7 buffer solution or freshly distilled water. If buffer solution is used, the beaker should be sufficiently identified that the "storing" solution will not be used to calibrate the instrument.